Cloning and functional analysis of type I and type II metacaspases during flower senescence in *Petunia x hybrida* cv. Mitchell Diploid

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Abstract

Senescence is a highly regulated process and the final stage of plant development, which ultimately results in the programmed death of cells, organs, tissues or whole plants. Caspases are key regulators of the cell death program in animals, but to date no homologs of caspases have been found in plant genome databases. While caspase-like activity has recently been demonstrated in various plant cell death models, the corresponding genes for these activities have never been identified. While a caspase related family of proteases (metacaspases) has been identified in plant and fungal genomes using iterative PSI-BLAST, the function of metacaspases in plants is still largely unknown. As a first step to understanding the role of metacaspases, a type I *(PhMCA1)* and a type II *(PhMCA2)* metacaspase have been cloned from *Petunia x hybrida* cv Mitchel Diploid. The expression of *PhMCA1* and *PhMCA2* has been investigated in various petunia tissues using real-time RT-PCR *PhMCA1* transcript abundance increased during flower senescence, while abundance of *PhMCA2* increased following *Botrytis cinerea* infection. *PhMCA1* expression was not increased in ethylene insensitive petunias during flower senescence. In order to further investigate the functional role of *PhMCA1,* RNAi lines have been generated and phenotypic analyses of T1 plants is underway.

Introduction

Programmed cell death (PCD) is a genetically controlled mechanism associated with plant morphology and the response to abiotic or biotic stresses. PCD in plants occurs during embryogenesis, xylem formation, hypersensitive response to biotic stresses and senescence. Senescence allows plants to remobilize nutrients from dying cells to actively growing tissues or organs. In animal PCD, cell death signaling pathways converge on a common death machinery that is activated by a series of cysteine proteinases (caspases). To date there has been no caspase homologue found in plant genomes. Structural homologues of caspases (metacaspases) have been found from plants, fungi and metazoan through iterative PSI-BLAST (Uren et al., 2000). In order to understand the functional role of

Results

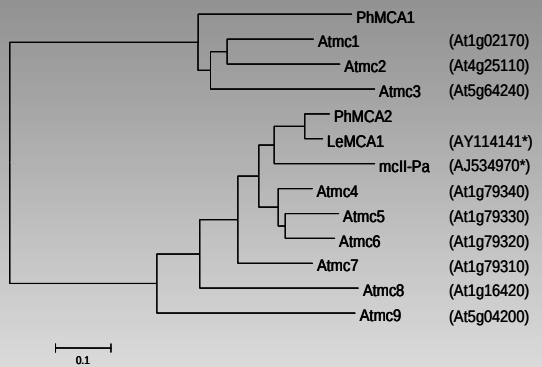

Fig. 1. Phylogenetic tree of the metacaspases of Norway spruce, tomato, Arabidopsis and petunia

The phylogenetic tree was constructed by MEGA3 software. Numbers in parentheses correspond to loci for Arabidopsis and Genbank accession numbers for Norway spruce (mcII-Pa) and tomato metacaspases (LeMCA1) indicated by *.

***PhMCA1* is a type I metacaspase and *PhMCA2* is a type II metacaspase.**

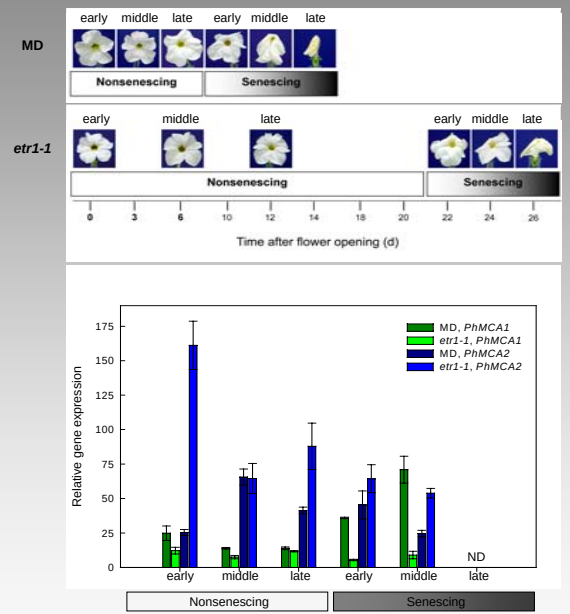

Figure 4. Relative expression of *PhMCA1* and *PhMCA2* in *Petunia x hybrida* cv. Mitchell Diploid (MD) and *etr1-1* during corolla senescence

metacaspases during flower senescence, a type I and a type II metacaspase were cloned from petunia and their expression in various organs, under biotic stress (*Botrytis cinerea* infection) and by ethylene was investigated.

Materials and Methods

Plant materials

Petunia x hybrida cv. Mitchell diploid and ethylene insensitive transgenic petunias (35S:*etr1-1*, line 44568) were grown in the greenhouse and used for all real time RT-PCR analysis.

Cloning of *PhMCA1* and *PhMCA2*

EST sequences of *PhMCA1* and *PhMCA2* were obtained from Computational Biology and Functional Genomics laboratory (http://compbio.dfci.cornell.edu/tgi/) and SOL genomics network (http://www.sgn.cornell.edu) websites. 5'and 3' RACE (Random Amplification of Complementary Ends) was performed to clone full length cDNAs using primers designed from the EST sequences. Sequencing was performed at the MCIC (Molecular Cellular Imaging Center) at OARDC.

Real time RT-PCR

Total RNA treated with RNase-free DNase (Promega) isolated from *Petunia x hybrida* cv. Mitchell Diploid corollas, anthers, ovaries, roots, leaves and stems was used to synthesize cDNA by reverse transcription reaction. The relative expression levels were analyzed and normalized to the petunia actin gene (*Phactin*).

***Botrytis cinerea* infection**

B. cinerea was cultured and inoculated as described by Benito et al. (1998) onto petunia leaves. Conidia were harvested from sporulating plates by washing with sterile water and conidial suspension was washed three times by centrifugation (8 min, 114 g) and resuspended in Gamborg's B5 basal medium, including vitamins, glucose (10mM), and sodium phosphate buffer (10 mM; pH 6.0). 8 week old leaves were removed from plants and petioles were inserted in microcentrifuge tubes filled with water. Tubes containing leaves were kept in a plastic box with high humidity. Leaves were inoculated with conidial suspensions, then leaves were dried at room temperature for 30 min. The plastic container was closed and incubated at 20ºC with a 16 h photoperiod.

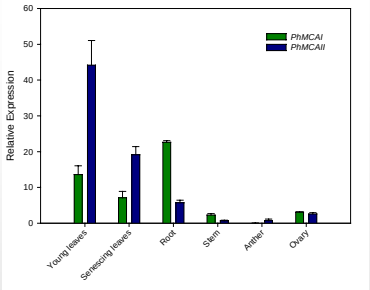

Figure 2. Relative expression of *PhMCA1* and *PhMCA2* in various petunia organs.

Total RNAs were isolated from various organs of petunia and real time RT-PCR analysis was performed as described in Materials and Methods. The data shown are means and standard errors.

Relative expression levels of *PhMCA1* and *PhMCA2* were higher in roots and leaves than in other organs tested. Expression of both metacaspases in senescing leaves was lower than in young leaves.

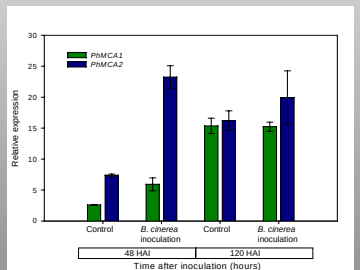

Figure 3. Relative expression of *PhMCA1* and *PhMCA2* following *B. cinerea* infection of petunia leaves.

Relative expression of *PhMCA1* and *PhMCA2* was measured and compared at 48 hours and 120 hours after inoculation (HAI) with *B. cinerea* and buffer by real time RT-PCR analysis as described in Materials and Methods. The data shown are means and standard errors. 10mM sodium phosphate buffer (pH6.0) was inoculated as a negative control.

***PhMCA2* transcript increased more than *PhMCA1* after *B. cinerea* infection.**

Relative expression levels of *PhMCA1* and *PhMCA2* were measured by real time RT-PCR as described in Materials and Methods. The data shown are means and standard errors. Corolla development stages and number of days after flower opening are indicated as shown. ND = not determined

Expression of *PhMCA1* was increased during corolla senescence in MD, but was low throughout corolla development in ethylene insensitive petunia (*etr1-1*). *PhMCA2* is expressed at the early stage of corolla development in both MD and *etr1-1*.

Conclusions

-A type I and a type II metacaspases were cloned and sequenced from *Petunia x hybrida cv*. Mitchell Diploid.

-Both metacaspases (*PhMCA1* and *PhMCA2*) contain the catalytic residues (His and Cys) that are highly conserved among caspases.

-The transcript abundance of the type I metacaspase (*PhMCA1*) increased during flower senescence, while the type II metacaspase (*PhMCA2*) was upregulated following *Botrytis cinerea* infection.

-Ethylene appeared to be required for the expression of *PhMCA1* and *PhMCA2* expression may be regulated by ethylene and additional signals during corolla senescence.

Literature Cited

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